

REMARKS***Status of the claims***

Claims 1-60 were pending in the present application. Claims 1-21 and 42-60 were withdrawn from consideration and remaining claims 22-41 were rejected. By virtue of this response, claims 1-21 and 42-60 have been cancelled, claims 22-31, 34-35, 38, and 40-41 have been amended, and new claims 61-88 have been added. Accordingly, claims 22-41 and 61-88 are currently under consideration.

With respect to all amendments and cancelled claims, Applicants have not dedicated or abandoned any unclaimed subject matter and, moreover, have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants reserve the right to pursue prosecution of any presently excluded claim embodiments in future continuation, continuation-in-part, and/or divisional applications.

Amendments to the Specification

The section entitled "Drawings" in the specification has been amended to more closely correspond to the labeling of the Figures as requested by the Examiner. No new matter is added.

Amendments to the Claims

Claims 1-21 and 42-60 have been cancelled, claims 22-31, 34-35, 38, and 40-41 have been amended, and new claims 61-88 have been added. No new matter is added.

Claim 22 is amended to explicitly state that both the attenuation of the bacterium for entry into non-phagocytic cells and the attenuation for cell-to-cell spread are relative to wild type. Support for this amendment is found, e.g., in paragraph [0080] at pages 18-19, lines 1-4 of paragraph [0082] at page 19, and lines 1-7 of paragraph [0125] at page 31.

Claim 22 is also amended to indicate that the non-phagocytic cells are hepatocytes. Support for this amendment is found, e.g., in paragraph [0087] at page 20-21 and lines 1-7 of paragraph [0109] at page 27.

Claim 23 is amended to explicitly state the relationship between the “at least one mutation” in the gene(s) and the attenuation of the bacterium for cell-to-cell spread recited in claim 22. Support for this amendment is found, e.g., in paragraph [0133] at page 34.

Claim 25 is amended to explicitly state the relationships between the modification of the nucleic acid of the bacterium, the attenuation of the bacterium for proliferation, and the attenuation of the bacterium for cell-to-cell spread recited in claim 22. Support for this amendment is found, e.g., in paragraph [0145] at page 38.

Claim 27 is amended to explicitly state the relationship between the defectiveness of the bacterium with respect to one or more internalins and the attenuation of the bacterium for entry into non-phagocytic cells recited in claim 22. Support for this amendment is found, e.g., in paragraphs [0096]-[0097] at pages 23-24. Claim 27 is further amended to explicitly state that the defectiveness is relative to wild type.

Claim 29 is amended to explicitly state the relationship between the “at least one mutation” and the attenuation of the bacterium for entry into non-phagocytic cells that is recited in claim 22. Support for this amendment is found, e.g., in paragraph [0098] at page 24.

Claim 30 is amended to explicitly state the relationship between the bacterium being “defective with respect to ActA” and the attenuation of the bacterium for cell-to-cell spread that is recited in claim 22. Support for this amendment is found, e.g., in paragraph [0133] at page 34.

Claim 31 is amended to explicitly state that “the mutation in *actA* attenuates the bacterium for cell-to-cell spread relative to wild type and the mutation in *inlB* attenuates the bacterium for entry into the non-phagocytic cells relative to wild type.” Support for this amendment is found, e.g.,

in paragraph [0028] at pages 8-9, paragraphs [0133]-[0135] at pages 34-35, and paragraphs [0096]-[0097] at pages 23-24.

Claim 38 has been amended to recite “composition” rather than “vaccine.” The reference to a pharmaceutically acceptable carrier has been deleted. Support is found, e.g., in paragraph [0176] at page 49.

The term “preventing” has been deleted from claim 40. Support is found, e.g., in the claim as originally filed.

The term “isolated” has been added to claim 41. Support is found, e.g., in lines 1-5 of paragraph [0011] at page 5.

In addition, minor amendments are made to claims 24, 26, 28, 34, and 35.

Support for new claims 61-88 is found, e.g., as indicated in Table 1 below. Support for the new method claims 61-82 is also found, e.g., in paragraph [0193] at page 55.

TABLE 1: EXEMPLARY SUPPORT FOR NEW CLAIMS

| NEW CLAIM NO. | SUPPORT IN SPECIFICATION AND/OR ORIGINAL CLAIMS |
|---------------|---|
| 61 | Lines 2-7 of paragraph [0133] at page 34; lines 3-5 of paragraph [0103] |
| 62 | Original claim 31; paragraph [0009] at pages 4-5 |
| 63 | Original claim 32; paragraph [0009] at pages 4-5 |
| 64 | Original claim 31; paragraph [0009] at pages 4-5 |
| 65 | Original claim 33; paragraph [0009] at pages 4-5; paragraph [0008] at pages 3-4 |
| 66 | Original claim 33; paragraph [0009] at pages 4-5; paragraph [0008] at pages 3-4 |
| 67 | Original claim 34; paragraph [0158] at page 43 |
| 68 | Original claim 36 |

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| 69 | Lines 2-7 of paragraph [0133] at page 34; lines 3-5 of paragraph [0103] |
| 70 | Original claim 31; paragraph [0009] at pages 4-5 |
| 71 | Original claim 32; paragraph [0009] at pages 4-5 |
| 72 | Original claim 31; paragraph [0009] at pages 4-5 |
| 73 | Original claim 33; paragraph [0009] at pages 4-5; paragraph [0008] at pages 3-4 |
| 74 | Original claim 33; paragraph [0009] at pages 4-5; paragraph [0008] at pages 3-4 |
| 75 | Original claim 34; paragraph [0158] at page 43 |
| 76 | Lines 10-14 of paragraph [0157] at page 42; paragraph [0009] at pages 4-5; paragraph [0202] at page 58 |
| 77 | Paragraph [0009] at pages 4-5; paragraph [0184] at pages 51-52; paragraphs [0187]-[0188] at pages 52-53 |
| 78 | Original claim 31; paragraph [0009] at pages 4-5 |
| 79 | Original claim 32; paragraph [0009] at pages 4-5 |
| 80 | Original claim 33; paragraph [0009] at pages 4-5; paragraph [0008] at pages 3-4 |
| 81 | Lines 10-14 of paragraph [0157] at page 42; paragraph [0009] at pages 4-5; paragraph [0202] at page 58 |
| 82 | Lines 10-14 of paragraph [0157] at page 42; paragraph [0009] at pages 4-5; paragraph [0202] at page 58 |
| 83 | Original claim 38 |
| 84 | Lines 1-7 of paragraph [0133] at page 34 |
| 85 | Lines 1-7 of paragraph [0133] at page 34; paragraphs [0134]-[0135] at page 35 |
| 86 | Paragraph [0110] at page 27; paragraph [0103] at page 25 |
| 87 | Paragraph [0028] at pages 8-9; paragraphs [0133]-[0135] at pages 34-35; paragraphs [0096]-[0097] at pages 23-24 |

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|----|---|
| 88 | Original claim 33; paragraph [0009] at pages 4-5; paragraph [0008] at pages 3-4 |
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Objection to the Specification

The Examiner has objected to the specification because the “Brief Description of Drawings” allegedly did not properly correspond to the figures. By virtue of this Amendment, the “Drawings” section in the specification has been amended to more closely correspond to the labeling of the Figures as requested by the Examiner. Accordingly, Applicants respectfully request that the objection to the specification be withdrawn.

Claim Objections

The Examiner has objected to claim 23 because the claim contains non-elected subject matter. The Examiner has required that the claim be amended to remove non-elected subject matter.

In response, Applicants respectfully submit that no amendment of claim 23 to remove non-elected subject matter should be required at this time. Claim 23 is a generic claim, and the previous election of a mutation in *actA* and *inlB* by Applicants in response to the Office Action mailed November 10, 2006 was a *species* election only.

As indicated in MPEP §821.02, “Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.” Thus, if a generic claim is found to be allowable, Applicants request consideration of an additional reasonable number of species not previously elected. If no generic claim is found to be allowable, but claims readable upon the elected species are found to be allowable with respect to the elected species, removal of non-elected species from the claims may be proper (see, e.g., MPEP 821.02). Since no final

determination as to the allowability of the claims has been made in this application, removal of the non-elected species from claim 23 at this time would be premature.

Accordingly, Applicants respectfully request that the objection to claim 23 as being drawn to non-elected subject matter be withdrawn.

Claim Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 22-41 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

1. Claim 22 was rejected for allegedly being vague and indefinite because it attempts to claim the bacterium by function alone. Applicants respectfully traverse this rejection.

Claim 22 is directed to an isolated *Listeria* bacterium which is attenuated both for entry into non-phagocytic cells and for cell-to-cell spread *relative to wild type*, wherein the non-phagocytic cells are hepatocytes.

Functional language in a claim is not improper per se. See MPEP 2173.05(g). A functional limitation must be evaluated and considered, just like any other limitation of the claim, for what it fairly conveys to a person of ordinary skill in the pertinent art in the context in which it is used. See MPEP 2173.05(g). Applicants contend that, contrary to the Examiner's assertions, the "metes and bounds" of claim 22, as amended, would be fully understood by one of ordinary skill in the art and one of ordinary skill in the art could readily identify the claimed bacteria. One of ordinary skill in the art would be readily able to identify whether a particular strain of *Listeria* was attenuated both for entry into non-phagocytic cells and attenuated for cell-to-cell spread relative to wild type *Listeria*, and, thus, the claim is not vague and indefinite.

Applicants contend that the meaning of the language in claim 22 that reads “attenuated...for entry into nonphagocytic cells” would be clear to one of ordinary skill in the art in view of the teachings of the specification. As indicated at lines 6-8 of paragraph [0085] of Applicants’ specification, “A *Listeria* bacterium that is attenuated for entry into non-phagocytic cells is less able to infect at least one type of non-phagocytic cell from the extracellular environment of the non-phagocytic cell than wild type *Listeria* of the same species.” A variety of non-limiting examples and descriptions of *Listeria* attenuated for entry into non-phagocytic cells are provided, e.g., from paragraph [0085] at page 20 to paragraph [0124] at page 31 of the specification. For instance, where the non-phagocytic cells are hepatocytes, as in the claims as amended, a non-limiting example of such a *Listeria* attenuated for this particular feature of entry into the non-phagocytic cells is *Listeria* in which the *inlB* gene has been deleted.

Furthermore, one of ordinary skill in the art would have a variety of *in vitro* and *in vivo* assays at their disposal for assessing whether certain *Listeria* are attenuated for entry into non-phagocytic cells relative to wild type. For instance, Applicants’ specification provides non-limiting, exemplary *in vitro* functional assays which can be used to assess whether *Listeria* are attenuated for entry into non-phagocytic cells. Examples of these assays are described, e.g., in paragraph [0089] at page 21, in paragraph [0092] at page 22, and Examples 9 and 10 at pages 76-78 of the specifications. In addition, based on Applicants’ teachings, one of ordinary skill in the art could also look to genetic assays in some instances to determine whether the *Listeria* are attenuated for entry into non-phagocytic cells. For example, one of ordinary skill in the art would be able to determine through sequencing if there was a significant disruption of the *inlB* gene in a strain of *Listeria monocytogenes*. A significant disruption of the *inlB* gene in the genome of a strain (e.g., a large deletion or frameshift mutation in the gene, etc.) would generally be expected to cause the *Listeria* to be attenuated for entry into non-phagocytic cells.

Similarly, Applicants contend that the meaning of the language in claim 22 that reads “attenuated...for cell-to-cell spread” would be clear to one of ordinary skill in the art in view of the teachings of the specification. As indicated at lines 3-5 of paragraph [0125] of Applicants’ specification, “A *Listeria* bacterium is attenuated for cell-to-cell spread if the *Listeria* bacterium is

less able to spread *intercellularly* from one infected cell (a cell comprising the *Listeria* within its cytoplasm) to a neighboring cell.” A variety of non-limiting examples of *Listeria* attenuated for entry into non-phagocytic cells are provided, e.g., from paragraph [0125] at page 30 to paragraph [0153] at page 41 of the specification. A non-limiting example of such a *Listeria* attenuated for this particular feature of cell-to-cell spread is *Listeria* in which the *actA* gene has been deleted.

One of ordinary skill in the art would likewise have a variety of *in vitro* and *in vivo* assays at their disposal for assessing whether certain *Listeria* are attenuated for cell-to-cell spread relative to wild type. For instance, Applicants’ specification provides non-limiting, exemplary *in vitro* assays for assessing whether *Listeria* are attenuated for cell-to-cell spread such as measuring plaque formation on cell monolayers (see, e.g., paragraph [0128] at pages 32 and 33). In addition, it has been well-established in the art that deletions of or inactivating mutations in certain genes such as *actA* will produce *Listeria* that are attenuated for cell-to-cell spread (see, e.g., Kocks et al., *Cell*, 68:521-531 (1992)). As a result, one of ordinary skill in the art will recognize that identification through sequencing of certain types of mutations (e.g., deletion, frameshift mutations, mutations in key active sites, etc.) in certain regions of the genome including, but not limited to, *actA*, would indicate that the *Listeria* are attenuated for cell-to-cell spread. Furthermore, paragraphs [0133]-[0139], at pages 34-37 of Applicants’ specification provide non-limiting examples of genes which, when significantly disrupted, can be expected to cause the *Listeria* to be attenuated for cell-to-cell spread.

2. Claim 25 was rejected because it was allegedly unclear “whether the ‘attenuation of the proliferation of the bacterium’ is distinct from the attenuation for cell-to-cell spread and/or entry into non-phagocytic cells as those characteristics encompass ‘proliferation’.” Applicants respectfully traverse this rejection and contend that the Examiner’s statement is not correct. Nevertheless, claim 25 has been amended by virtue of this Amendment to explicitly state the relationships between the modification of the nucleic acid of the bacterium, the attenuation of the bacterium for proliferation, and the attenuation of the bacterium for cell-to-cell spread recited in claim 22. Accordingly, Applicants respectfully request that the rejection of claim 25 be withdrawn.

3. The Examiner has asserted that claim 27 is vague and indefinite because it is unclear what is encompassed by the phrase “defective with respect to one or more internalins.” Applicants respectfully traverse this rejection.

Applicants contend that the phrase in question would have been neither vague nor indefinite to one of ordinary skill in the art, especially in light of Applicants’ specification.

One of ordinary skill in the art would recognize from the specification that a bacterium said to be defective with respect to a particular protein, is defective with respect to that protein relative to wild type or another bacterium of the same species without the defect. Claim 27 has now been amended to recite “relative to wild type” explicitly in the claim. Claim 27 has also been amended to explicitly state that a consequence of the bacterium being defective with respect to one or more internalins is that the bacterium is attenuated for entry into the non-phagocytic cells.

In addition, the specification indicates that bacteria are “defective with respect to” an invasin protein such as an internalin if they either (a) produce decreased amounts of a functional version of the invasin protein (relative to wild type) or (b) express a version of the invasin protein that is partially or totally nonfunctional (relative to wild type), or both. See, e.g., paragraphs [0096]-[0097] at page 24. See also paragraph [0110] at page 27 of the specification, regarding bacteria defective with respect to internalin B.

In view of the above, Applicants respectfully request that the rejection of claim 27 be withdrawn.

4. Claim 29 also stands rejected as allegedly being vague and indefinite because the claim does not describe either the location/type of mutation or the consequence of the mutation. Applicants respectfully traverse this rejection.

Claim 29 has now been amended to expressly state that the bacterium “comprises at least one mutation in the *inlB* gene that attenuates the bacterium for entry into the non-phagocytic cells.” Applicants contend that the claim, as amended, recites both the location of the mutation (i.e., in the

inlB gene), as well as the type and consequence of the mutation (i.e., a mutation that attenuates the bacterium for entry into non-phagocytic cells.” Accordingly, Applicants respectfully request that the rejection of claim 29 be withdrawn.

5. The Examiner has asserted that claim 30 is vague and indefinite because it is unclear what is encompassed by the phrase “defective with respect to ActA.” Applicants respectfully traverse this rejection.

Applicants contend that the phrase in question would have been neither vague nor indefinite to one of ordinary skill in the art, especially in light of Applicants’ specification. One of ordinary skill in the art would have recognized that a bacterium said to be defective with respect to a particular protein, is defective with respect to that protein relative to wild type. Claim 30 has now been amended to recite “relative to wild type” explicitly in the claim. Bacteria are “defective with respect to” a particular protein if they either (a) produce decreased amounts of a functional version of the protein (relative to wild type) or (b) express a version of the protein B that is partially or totally nonfunctional (relative to wild type), or both. See, e.g., paragraph [0097] at page 24 and paragraph [0110] at page 27 of the specification regarding bacteria defective with respect to other types of proteins such as internalin B.

6. Claim 31 is rejected for being vague and confusing because it is unclear what function the mutation causes. Applicants respectfully traverse. Nevertheless, in the interest of expediting prosecution, Applicants have amended claim 31 to more clearly state that the mutation in *actA* attenuates the bacterium for cell-to-cell spread and the mutation in *inlB* attenuates the bacterium for entry into the non-phagocytic cells. Accordingly, Applicants respectfully request that the rejection of claim 31 be withdrawn.

7. Regarding claim 39, the Examiner has alleged that there is insufficient antecedent basis for “the antigen” in the claim. Applicants traverse. Applicants note that reference is made to “an antigen” in the first line of claim 39. Applicants therefore believe that proper antecedent basis for “the antigen” in claim 30 already exists.

8. Regarding claim 41, the Examiner has indicated that the claim should be amended to recite that the professional antigen-presenting cell has been “isolated.” In response, Applicants have amended claim 41 per the Examiner’s suggestion.

In light of the above remarks, Applicants respectfully request that the rejection of claims 22-41 under 35 U.S.C. § 112, second paragraph, be withdrawn.

Provisional Nonstatutory Obviousness-Type Double Patenting Rejections

1. Claims 22-38 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-122 of copending Application No. 10/883,599. In response, Applicants note that this is a provisional rejection only. Applicants will address this rejection, if maintained, at the appropriate time if conflicting claims are found allowable.

2. Claims 39 and 40 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 20-149 of copending Application No. 10/773,618. In response, Applicants note that this is a provisional rejection only. Applicants will address this rejection, if maintained, at the appropriate time if conflicting claims are found allowable.

Claim Rejections under 35 U.S.C. § 112, First Paragraph

1. Enablement

Claims 22-41 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. Applicants respectfully traverse this rejection.

The basis for the Examiner’s written description rejection appears to be essentially three-fold:

A. The specification allegedly does not enable either prevention or protection.

- B. The specification allegedly does not enable a number of mutations in *actA* and *inlB* resulting in attenuation for entry into non-phagocytic cells and cell-to-cell spread that is commensurate with the scope of the claims.
- C. The specification allegedly does not enable *actA* and *inlB* mutants that belong to species of *Listeria* other than *L. monocytogenes*.

Applicants respectfully traverse this rejection and address each of the above-listed assertions by the Examiner in turn below.

- A. *The full scope of the claims with respect to both methods of treating and protecting against disease is enabled.*

The Examiner has asserted that the “specification has not taught (through challenge experiments) that this bacterium may provide protection (vaccine) or prevent infections caused by *Listeria*. Treatment [*sic*] of *Listeria monocytogenes* infection has been enabled, but not prevention or protection.” See page 7 of Office Action. Although Applicants appreciate the statement by the Examiner that treatment of infections caused by *Listeria monocytogenes* with the compositions of the invention have been enabled, Applicants respectfully contend that the full scope of the claims is enabled, including treatment of diseases other than those caused by *Listeria*, such as cancer, as well as prevention and protection of diseases.

Applicants assert that the term “vaccines” is recognized by those of ordinary skill in the art to be a term that encompasses compositions that are capable of preventing or protecting against disease, as well as compositions that are therapeutic in nature. In addition, the specification of the present application clearly indicates that the term “vaccine” is intended to encompass both a prophylactic vaccine, as well as a therapeutic vaccine. See, e.g., paragraph [0184] at pages 51-52 of Applicants’ specification. Nevertheless, in the interest of expediting prosecution, claim 38 has now been amended, without prejudice, to remove reference to “vaccine.” Also, claim 40 has been amended to remove the reference to “preventing” disease. (Newly added method claims 77-82, however, are directed to methods of providing *protection* against a disease.)

Applicants' own specification provides working examples which demonstrate the ability of an *actAinlB* double deletion mutant strain of *Listeria monocytogenes* to induce an immune response against an antigen, to treat tumors in an *in vivo* mouse model, and to provide protection against cancer in an *in vivo* mouse model. The immunogenicity of an *actAinlB* double deletion mutant strain of *Listeria monocytogenes* is demonstrated in Applicants own specification, e.g., in Example 4 (*in vivo* cytotoxicity) and Example 7 (measurement of CD8+ model antigen-specific T-cells by Intracellular Cytokine Staining (ICS)) in the specification. The ability of an *actAinlB* double deletion mutant strain of *Listeria monocytogenes* to treat tumor-bearing mice is also demonstrated in Example 4 (p. 70-71) and Figures 2A-C of the application. The *actAinlB* mutant *Listeria* used in Example 4 which expressed a model antigen, AH1-A5, were found to decrease the number of lung metastases that developed in the mice in which tumor cells expressing the MMTV gp70 epitope AH1 had been previously implanted (Figure 2A) and to significantly increase survivability of the mice (Figures 2B-C).

Furthermore, additional data supporting the enablement of the methods of using the claimed compositions to induce immune responses, to treat cancer, and to provide protection against cancer or other disease conditions can be found, e.g., in other publications. For instance, see U.S. Patent Publication No. 2005/0249748. Paragraphs [0612] to [0616] (Example 31D) and Figure 64 of this patent publication provide data indicating that an *actAinlB* double deletion mutant of *Listeria monocytogenes* that had been engineered to express human mesothelin, a tumor antigen, can slow the growth of a tumors in mice that had previously been implanted with tumor cells that expressed human mesothelin. Thus, as articulated in paragraph [0616] of the publication, the mutant *Listeria* was shown to be protective. Paragraphs [0603] to [0606] (Example 31B) and Figure 61 of the publication presents results that show the therapeutic efficacy of an *actAinlB* *Listeria* mutant. As shown in Figure 61 of the publication, *Listeria actAinlB* double mutants that expressed human mesothelin were found to prolong survival in tumor-bearing mice where the tumor cells in the mice had been engineered to express human mesothelin. Similarly, the data in paragraphs [0607] to [0609] (Example 31C) and Figure 62 of the specification demonstrates the ability of *Listeria actAinlB* double mutants to reduce the number of lung tumor nodules that develop in mice that had

previously been injected with tumor cells that had been engineered to express human mesothelin. In addition, evidence of the ability of *actAinlB* mutants to protect against a subsequent challenge is provided in Table 1 of the Supporting Information available online at www.pnas.org/cgi/content/full/0406035101/DC1 for the journal article Brockstedt et al., "Listeria-based cancer vaccines that segregate immunogenicity from toxicity," *PNAS*, 101:13832-13837 (2004). Table 1 of the Supporting Information indicates that immunization with 0.1 LD₅₀ of the *actAinlB* *L. monocytogenes* mutant "CS-L0001," elicits protective immunity (see "Log protection" of Table 1) against subsequent challenge with 2 x LD₅₀ wild type *L. monocytogenes* 28 days post the primary vaccination.

Thus, it is believed that the pending claims are fully enabled for both treatment of disease and for protection against disease.

B. The full scope the claims with respect to actA and inlB mutations is enabled.

The Examiner has stated on page 8 of the Office Action, "Knowledge of the sequence of protein or polynucleotide alone is not sufficient for those skilled in the art to make any mutation to a molecule and have confidence as to the effects that such a mutation would have." Later, at page 9 of the Office Action, the Examiner states, "The specification does not provide evidence that one skilled in the art would know what modifications, and what regions of *inlB* and *actA* to target for modifications, in order to produce an attenuated bacterium with the desired phenotype." Applicants, however, contend that adequate direction and guidance is provided in Applicants' specification to enable one of ordinary skill in the art to make and/or use the full scope of the claimed invention, including a wide, representative variety of *actA* and *inlB* mutants.

The enablement requirement of 35 U.S.C. § 112, paragraph 1, requires that the claimed invention be enabled so that any person skilled in the art can make and use the invention without undue experimentation. *In re Wands*, 858 F. 2d at 737, 8 USPQ2d at 1404 (Fed. Cir. 1988). The test of enablement is not whether any experimentation is necessary but whether if experimentation is necessary, it is undue. *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976).

Furthermore, as stated in MPEP §2164.01, “A patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987); and *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984).”

Applicants respectfully submit that the specification, in light of the knowledge of those of ordinary skill in the art at the time, provides a more than adequate disclosure to enable one of ordinary skill in the art to make and use a wide range of *actA* and *inlB* *Listeria* mutants.

The *Listeria* of pending claim 22 may be attenuated for entry into hepatocytes by any one of various routes that are readily attainable by one of ordinary skill in the art without any undue experimentation. The specification highlights that one such method is by altering the strain such that it is defective with respect to internalin B. As described by Applicants specification, this may be effected, e.g., by disruption of the coding sequence (see, e.g., paragraphs [0110] and [0112]) and/or by inhibition of expression of internalin B (see, e.g., paragraphs [0110] and [0113]). As indicated in paragraph [0111] of Applicants specification, a mutation in the *inlB* gene may be a mutation such as a point mutation, an insertion mutation, a termination mutation, a frame shift mutation, or a deletion of part or whole of the gene encoding the internalin B. As indicated in paragraph [0113], in some embodiments, the mutation may be in one or more of the control sequences (such as the promoter or ribosome binding region) of *inlB*, so that expression is deleted or eliminated. One of ordinary skill in the art would be readily able to generate, for example, mutants in which a significant part or all of the *inlB* gene was deleted, mutants in which a stop codon had been placed early in the *inlB* coding sequence, or mutants in which a insertion mutation causes a frame shift in the internalin B coding sequence.

(Applicants note that the Examiner has stated, “It is disclosed that the function of the mutated gene may be from 25-100% less than the protein produced from a non-mutated gene sequence.” Applicants respectfully submit that this statement is not entirely correct and that some, but not all, *Listeria* in the claims comprise mutations having such a level of function.)

It is significant that if an *inlB* mutant of *Listeria* attenuated for entry into hepatocytes is sought, the expression or activity of the *inlB* gene product is being *disrupted* by the mutation(s). Although it may be more of a challenge to identify mutant forms of a protein that maintain function or have improved function, that is not what is required here. Generating functional mutants of a protein can be difficult precisely because it is so easy to render a protein nonfunctional once the sequence is known, even if structural information about the protein is not known. For instance, a frame-shift mutation is going to disrupt any protein's function, regardless of whether or not the structure-function relationship of the protein is known. Likewise, it is generally routine to eliminate expression of the protein once the gene sequence has been identified. One does not need any special information about the protein such as "which portions of the ... *inlB* gene are necessary for entry into non-phagocytic cells" (page 9 of the Office Action) to know that deletion of the gene, a frame-shift mutation in the coding sequence, or an inserted stop codon early in the coding sequence is going to disrupt expression. Even many of the possible point mutations that could be generated would be expected to disrupt the production of internalin B, regardless of the specifics of the protein structure. With respect to point mutations, Griffiths et al. states that, "it is always true that such mutations are more likely to reduce or eliminate gene function (thus they are loss-of-function mutations) than to enhance it. The reason is simple: it is much easier to break a machine than to alter the way that it works by randomly changing or removing one of its components." (page 315; Griffiths, et al. (2002) *Modern Genetic Analysis*, W.H. Freeman and Co., New York, NY).

Although *inlB* genes in species other than *L. monocytogenes* will generally be expected to differ somewhat in sequence from the *inlB* gene in *L. monocytogenes*, this doesn't change the fact that disrupting the expression or functionality of the protein in that other species will likewise be routine. If the gene has been identified, its expression or sequence can generally be readily disrupted, regardless of how much information is known about the details of the protein.

To confirm that a particular mutant *Listeria* strain is, in fact, attenuated for entry into hepatocytes, one of ordinary skill in the art would be able to use *in vitro* and *in vivo* assays taught in the specification and/or otherwise known to those of ordinary skill in the art. The ability to infect hepatocytes can be measured directly *in vitro*. Teachings and examples regarding such *in vitro*

assays can be found, e.g., at paragraphs [0089] and [0092] and in Examples 9 and 10 of Applicants' specification. *In vivo* methods of assaying for a decreased ability to enter non-phagocytic cells are provided in paragraphs [0093] to [0094], as well as in Example 8.

Similarly, Applicants contend that methods for attenuating *Listeria* for cell-to-cell spread, would also be routine to one of ordinary skill in the art in light of Applicants' disclosure. Applicants provide guidance regarding a number of genes which may be targeted to produce *Listeria* attenuated for cell-to-cell spread (relative to wild type). See, e.g., paragraphs [0133] to [0140]. Guidance regarding methods of producing the mutants are provided e.g., in paragraphs [0141] to [0142] at pages 38-39 of the specification. As discussed above with respect to *inlB* mutations, one of ordinary skill in the art would be readily able to mutate a target gene so as to disrupt the functionality of its gene product and/or expression of that gene product. For instance, it would be readily apparent to one of ordinary skill in the art that deleting most or all of the gene in question, causing a frame shift mutation, or generating a termination mutation would all be likely to produce the desired effect. In addition, Skoble et al, J. Cell Biology, 150:527-537 (2000) describes a variety ActA mutants and the effects of such mutations on actin polymerization (a function very important for cell-to-cell spread).

Furthermore, assays for confirming that a given mutant is, in fact, attenuated for cell-to-cell spread are provided in Applicants' specification and are known to those of ordinary skill in the art. For instance, Applicants' specification provides non-limiting, exemplary *in vitro* assays for assessing whether *Listeria* are attenuated for cell-to-cell spread such as measuring plaque formation on cell monolayers (see, e.g., paragraph [0129] at pages 32 and 33). Assays for assessing the effect of mutations in ActA on actin polymerization *in vitro* and *in vivo* were known in the art as demonstrated in Skoble et al, J. Cell Biology, 150:527-537 (2000).

Applicants respectfully submit that in light of Applicants' teachings it would be routine for one of ordinary skill in the art to follow the guidelines set out in Applicants' specification in light of the knowledge of those of ordinary skill in the art to generate a variety of *actA* and *inlB* mutants,

other mutants, or other modified forms of *Listeria* that are attenuated for entry into hepatocytes and for cell-to-cell spread.

C. *The full scope of the claims with respect to species other than just L. monocytogenes is enabled.*

The Examiner has stated, “The claims are broadly drawn to obtaining attenuated *Listeria* bacteria by mutating variant nucleotide sequences from many different species of *Listeria* yet the specification has only taught mutation of the *actA* and *inlB* genes from *L. monocytogenes*. In the present case, the applicant has neither provided any direction or guidance, nor any working examples in the specification as to any potential mutations of *actA* and *inlB* genes from other species of *Listeria* that would satisfy the limitations of the claims.” Applicants respectfully disagree.

Applicants contend that adequate direction and guidance is provided in Applicants specification to enable one of ordinary skill in the art to make and/or use the full scope of the claimed invention, including *Listeria* belonging to species other than *L. monocytogenes*. Applicants contend that *actA* and *inlB* genes had been identified in a representative number of *Listeria* species at the date of filing the patent application, sufficient assays were provided in Applicants specification and/or were known to those of ordinary skill in the art to effectively screen for relevant mutants without undue experimentation, and some methods of attenuating *Listeria* for cell-to-cell spread or entry into non-phagocytic cells that are taught in Applicants specification are independent of the knowledge of the sequences of specific genes whose expression products are involved in cell-to-cell spread or entry into non-phagocytic cells.

Genes encoding internalin B mutants have not only been identified for *Listeria monocytogenes*, but also for other *Listeria* as well. Information regarding the sequence of *inlB* in *Listeria monocytogenes* is provided e.g., in paragraph [0221] at pages 61-62, of the present application. In addition, the *i-inlB2* gene encoding an internalin B protein had been identified in

Listeria ivanovii prior to the filing date of the present application. See, e.g., Genbank Accession No. AJ271621.

In addition to those genes which had been identified in *Listeria* species at the time of filing, one of ordinary skill in the art would have been able to readily identify mutations in other genes in any given particular species by testing mutants for the desired phenotype, such as an attenuated ability to enter non-phagocytic cells such as hepatocytes relative to wild type, using *in vitro* and *in vivo* assays taught in the specification and/or otherwise known to those of ordinary skill in the art. For instance, the ability to infect hepatocytes can be measured directly *in vitro*. Teachings and examples regarding such *in vitro* assays can be found, e.g., at paragraphs [0089] and [0092] and in Examples 9 and 10 of Applicants' specification. *In vivo* methods of assaying for a decreased ability to enter non-phagocytic cells are provided in paragraphs [0093] to [0094], as well as in Example 8.

In addition, Applicants have provided at least one method of modifying *Listeria* to decrease its ability to enter non-phagocytic cells that is not dependent upon the mutation of particular sequences within the *Listeria* and is therefore independent with respect to the particular species to which the *Listeria* belongs. This method for attenuating the *Listeria* for entry into non-phagocytic cells, opsonization, is taught, e.g., at pages 30-31, paragraphs [0120] to [0124], and Example 10 at pages 77-78, paragraphs [0254] to [0255]. The experiments presented in Example 10 and Figure 9 show that an opsonized *actA* deletion mutant has a reduced ability to infect a non-phagocytic cell line comparable to that of *Listeria* deleted for both *actA* and *inlB*.

Not only would one of ordinary skill in the art have been able to adapt Applicants' teachings to decrease the ability of *Listeria* belonging to species other than *L. monocytogenes* for entry into non-phagocytic cells, but also one of ordinary skill in the art would have been able to adapt Applicants' teachings to decrease the ability of *Listeria* of other species for cell-to-cell spread. The *actA* gene, for instance, had been identified in more species of *Listeria* than just *L. monocytogenes*. Information regarding the *actA* gene in *L. monocytogenes* is provided in Applicants' specification. See also Kocks, et al., *Cell*, 68:521-531 (1992) and Genbank accession no. AL591974. The *actA*

gene for *Listeria ivanovii* had also been identified at the time of filing. See, e.g., Genbank accession no. X81135.

In addition, one of ordinary skill in the art would likewise have had a variety of routine *in vitro* and *in vivo* assays at their disposal for assessing whether certain mutant *Listeria* are attenuated for cell-to-cell spread. For instance, Applicants' specification provides non-limiting, exemplary *in vitro* assays for assessing whether *Listeria* are attenuated for cell-to-cell spread such as measuring plaque formation on cell monolayers (see, e.g., paragraph [0128] at pages 32 and 33). Additional information regarding assays for confirming that a given mutant is, in fact, attenuated for cell-to-cell spread are provided in paragraphs [0129] to [0131] at pages 32 to 34 and are known to those of ordinary skill in the art.

Furthermore, Applicants have provided methods of attenuating *Listeria* for cell-to-cell spread which are independent of knowledge of or mutation of genes that encode proteins that are involved in cell-to-cell spread. See, e.g., paragraphs [0145]-[0151] and [0153] at pages 38-41 of the specification. See, also, Example 11 at pages 78-81 of the specification. For instance, in some embodiments, the nucleic acid of the *Listeria* has been modified by reaction with a nucleic acid targeting compound so that proliferation of the bacterium is attenuated, thereby attenuating the bacterium for cell-to-cell spread. In such embodiments, the *Listeria* may be mutated so that it is defective with respect to a protein that functions to repair modifications to the *Listeria's* nucleic acid, but the treatment of the *Listeria* with the nucleic acid targeting compound itself (e.g., a crosslinking compound such as psoralen) will often be wholly independent of the specific gene sequences of that species of *Listeria*.

In light of above remarks, Applicants respectfully request that the rejection of claims 22-41 under 35 U.S.C. § 112, first paragraph as failing to comply with the enablement requirement be withdrawn.

2. Written description

Claims 22-41 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. Applicants respectfully traverse this rejection.

The basis for the Examiner's written description rejection appears to be two-fold:

- A. The specification allegedly does not provide adequate written description to support species homologs to *L. monocytogenes actA* and *inlB* genes.
- B. The specification allegedly does not provide adequate written description to support the scope of mutations.

Applicants respectfully traverse this rejection and address both of the above-listed assertions made by the Examiner in turn below.

- A. *The application provides adequate written description to support the full scope of the claims with respect to species other than L. monocytogenes.*

As support for her assertion that the specification does not provide adequate written description to support species homologs to *L. monocytogenes actA* and *inlB* genes, the Examiner states on page 11 of the Office Action, "The applicant has not identified any common structural core which one skilled in the art could use to identify any genus of polynucleotides to be mutated. In essence, the applicant is claiming such mutants comprising homologues only by their functionality, that of attenuated cell-to-cell spread and entry into non-phagocytic cells." The Examiner further asserts that the Federal Circuit has held that claiming polynucleotides disclosed by their biological function alone is inadequate to meet the written description requirement, citing *Amgen, Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016 (Fed. Cir. 1991) and *Regents of the Univ. of Cal. v. Eli Lilly and Co.*, 43 USPQ2d 1398 (Fed. Cir. 1997), and that the Federal Circuit case law supports her position regarding Applicants' claims.

Applicants respectfully traverse this rejection and contend that the Examiner has misapplied the law to Applicants' claims.

It is well established that to meet the written description requirement, an applicant's specification must "convey to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention." *Vas-Cath, Inc. v. Marhurkar*, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). This is the standard that the Federal Circuit has set. The portions of both of the opinions, *Amgen* and *Eli Lilly*, that were cited by the Examiner concerned claims directed to polynucleotides where the sequences of a representative number of the claimed polynucleotides were not previously known in the art. In a subsequent Federal Circuit opinion, *Falkner v. Inglis*, which concerned claims directed to vaccines comprising mutant viral sequences, claims that are more analogous to those of the present application than the claims that were the subject of either *Amgen* or *Eli Lilly*, the Federal Circuit clearly stated that "(1) examples are not necessary to support the adequacy of a written description (2) the written description standard may be met...even where actual reduction to practice of an invention is absent; and (3) *there is no per se rule that an adequate written description of an invention that involves a biological macromolecule must contain a recitation of a known structure.*" (emphasis added) 448 F.3d 1357, 1366, 79 U.S.P.Q.2d 1001, 1007 (Fed. Cir. 2006). See also, e.g., MPEP 2163(II)(A)(3)(a). As indicated in MPEP 2163(II)(A)(2), generally "there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. Information which is well known in the art need not be described in detail in the specification. See, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.* 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986)." Furthermore, in *Falkner v. Inglis*, the Federal Circuit held that "where...accessible literature sources clearly provided as of the relevant date, genes and their nucleotide sequences..., satisfaction of the written description requirement does not require either the recitation or incorporation by reference...of such genes and sequences." 448 F.3d 1357, 1368 (Fed. Cir. 2006).

Applicants contend that one of ordinary skill in the art would have readily recognized that Applicants were in possession of the invention as claimed in claim 22 and the dependent claims. In support, Applicants submit that (a) *actA* and *inlB* genes had been identified in a representative number of *Listeria* species at the time of filing and (b) some methods of attenuating *Listeria* for

cell-to-cell spread or entry into non-phagocytic cells that are taught in Applicants specification are independent of the knowledge of the actual sequences of specific genes whose expression products are involved in cell-to-cell spread or entry into non-phagocytic cells.

Genes encoding an internalin B protein had been identified for more than one *Listeria* species at the time of filing. Information regarding the sequence of *inlB*, in *Listeria monocytogenes* is provided e.g., in paragraph [0221] at pages 61-62, of the present application. In addition, the *i-inlB2* gene encoding an internalin B protein had been identified in *Listeria ivanovii* prior to the filing date of the present application. See, e.g., Genbank Accession No. AJ271621 (first appearing on Jan. 2003).

In addition, Applicants have provided at least one method of modifying *Listeria* to decrease its ability to enter non-phagocytic cells that is not dependent upon the mutation of particular sequences within the *Listeria* and is therefore independent with respect to the particular species to which the *Listeria* belongs. This method for attenuating the *Listeria* for entry into non-phagocytic cells, opsonization, is taught, e.g., at pages 30-31, paragraphs [0120] to [0124], and Example 10 at pages 77-78, paragraphs [0254] to [0255]. The experiments presented in Example 10 and Figure 9 show that an opsonized *actA* deletion mutant has a reduced ability to infect a non-phagocytic cell line comparable to that of *Listeria* deleted for both *actA* and *inlB*.

Not only would one of ordinary skill in the art have recognized that Applicants' teachings regarding the attenuation of *Listeria* for entry into non-phagocytic cells are adaptable to species other than just *L. monocytogenes*, but one of ordinary skill in the art would also have recognized that Applicants' teachings regarding the attenuation of *Listeria* for cell-to-cell spread are also adaptable to species other than just *L. monocytogenes*. The *actA* gene, for instance, had been identified in more species of *Listeria* than just *L. monocytogenes*. Information regarding the *actA* gene in *L. monocytogenes* is provided in Applicants' specification. See also Kocks, et al., *Cell*, 68:521-531 (1992) and Genbank accession no. AL591974. The *actA* gene for *Listeria ivanovii* had also been identified at the time of filing. See, e.g., Genbank accession no. X81135.

Even in those instances where mutation of a gene other than *actA* was desired in *Listeria monocytogenes* or *Listeria* belonging to another species, one of ordinary skill in the art would likewise have recognized that a variety of *in vitro* and *in vivo* assays were at their and Applicants' disposal for assessing whether certain mutant *Listeria* are attenuated for cell-to-cell spread. For instance, Applicants' specification provides non-limiting, exemplary *in vitro* assays for assessing whether *Listeria* are attenuated for cell-to-cell spread such as measuring plaque formation on cell monolayers (see, e.g., paragraph [0128] at pages 32 and 33). Additional information regarding assays for confirming that a given mutant is, in fact, attenuated for cell-to-cell spread are provided in paragraphs [0129] to [0131] at pages 32 to 34 and are known to those of ordinary skill in the art.

Furthermore, Applicants note that they have provided methods of attenuating *Listeria* for cell-to-cell spread which one of ordinary skill in the art would recognize as being independent of knowledge of or mutation of genes that encode proteins that are involved in cell-to-cell spread. See, e.g., paragraphs [0145]-[0151] and [0153] at pages 38-41 of the specification. See, also, Example 11 at pages 78-81 of the specification. For instance, in some embodiments, the nucleic acid of the *Listeria* has been modified by reaction with a nucleic acid targeting compound so that proliferation of the bacterium is attenuated, thereby attenuating the bacterium for cell-to-cell spread. In such embodiments, the *Listeria* may be mutated so that it is defective with respect to a protein that functions to repair modifications to the *Listeria's* nucleic acid, but the treatment of the *Listeria* with the nucleic acid targeting compound itself (e.g., a crosslinking compound such as psoralen) will often be wholly independent of the specific gene sequences of that species of *Listeria*. Therefore, one of ordinary skill in the art would have recognized that even if there was an absence of knowledge in the art about the sequences of particular genes involved in cell-to-cell spread in a particular species of *Listeria*, Applicants would nonetheless have been in possession of methods of attenuating the *Listeria* for cell-to-cell spread for a representative number of species.

B. The application provides adequate written description to support the full scope of the claims with respect to attenuating mutations.

The Examiner asserts in the Office Action that the “specification does not provide evidence that one skilled in the art would know what modifications, and what regions of the *inlB* and *actA* coding regions to target for modifications, in order to produce an attenuated bacterium.” See page 12 of the Office Action. The Examiner further asserts that “one skilled in the art would not be able to recognize from the current disclosure any substitutions, or other mutation (except, perhaps, deletion of the whole polynucleotide) that would result in a decreased gene product activity.” See page 13 of the Office Action. The Examiner cites the reference, Bowie et al., in support of her assertions.

Applicants respectfully traverse and contend that the Bowie et al. reference does not support the Examiner’s assertions. Furthermore, Applicants also respectfully submit that the specification of the present application does provide a representative number of species regarding the types of mutations that is sufficient to support a genus claim, and, further, that a representative number need not be large in light of the knowledge of those of skill in the art. In light of the knowledge of those of skill in the art in this area, those of skill in the art would have recognized from Applicants’ specification that Applicants were, in fact, in possession of the full scope of the invention as claimed.

Applicants respectfully contend that the Bowie et al. reference is largely irrelevant to the present application. The Bowie et al. reference describes “how an analysis of allowed amino acid substitutions in proteins can be used to reduce the complexity of sequences and reveal important aspects of structure and function.” See first paragraph on page 1306 of Bowie et al. The Examiner has pointed to nothing in the reference which would indicate that one of ordinary skill in the art would not readily envision multiple different ways that could be used to *disrupt* the expression or functionality of a given sequence. Even if it is true that one of ordinary skill in the art may often have trouble making amino acid substitutions in a particular protein sequence while still maintaining functionality, this is irrelevant to the present application. Unpredictability in making amino acid substitutions in a protein in which the structure-function relationship is unclear does not translate into there being unpredictability in the ability to *disrupt* the expression or function of a sequence. Regardless of how mysterious the structure-function relationships are, it would be obvious to one of

ordinary skill in the art that disruption of expression and/or function of a gene would most likely occur if certain things are done, such as, but not limited to, any of the following: (a) deletion of the entire coding sequence; (b) deletion of the majority of the coding sequence; (c) generation of one or more stop codons early in the coding sequence; (d) a deletion early in the coding sequence that generates a frame-shift mutation (e) an insertion early in the coding sequence that generates a frame-shift mutation; (f) deletion of the promoter or other key control sequence; and (g) deletion of both the promoter and the coding sequence of the gene. The effect of these types of mutations is far more predictable than the effect of the types of individual amino acid substitutions such as those discussed in Bowie et al.

Furthermore, even if single point mutations in a gene are being made, one skilled in the art would recognize that such mutations would be more likely to disrupt the function of the gene than not. As noted above, with respect to point mutations, Griffiths et al. states that, "it is always true that such mutations are more likely to reduce or eliminate gene function (thus they are loss-of-function mutations) than to enhance it. The reason is simple: it is much easier to break a machine than to alter the way that it works by randomly changing or removing one of its components." (page 315; Griffiths, et al. (2002) Modern Genetic Analysis, W.H. Freeman and Co., New York, NY).

Applicants contend that sufficient disclosure is provided in Applicants specification to show possession of a range of mutations having the desired claimed effects. One of ordinary skill in the art would readily recognize that the inventors were in possession of such a genus of mutations because the specification teaches that the *Listeria* may be attenuated for entry into hepatocytes by various routes, a representative number of which are provided. The specification highlights that one such method is by altering the strain such that it is defective with respect to internalin B. As described by Applicants specification, this may be effected, e.g., by disruption of the coding sequence (see, e.g., paragraphs [0110] and [0112]) and/or by inhibition of expression of internalin B (see, e.g., paragraphs [0110] and [0113]). As indicated in paragraph [0111] of Applicants specification, a mutation in the *inlB* gene may be a mutation such as a point mutation, an insertion mutation, a termination mutation, a frame shift mutation, or a deletion of part or whole of the gene encoding the internalin B. As indicated in paragraph [0113], in some embodiments, the mutation

may be in one or more of the control sequences (such as the promoter or ribosome binding region) of *inlB*, so that expression is deleted or eliminated. Based on the sequence information taught about *inlB* (see, e.g., paragraph [0109] at page 27), one of ordinary skill in the art would view the inventors as being in possession of, e.g., mutants in which a significant part or all of the *inlB* gene was deleted, mutants in which a stop codon had been placed early in the *inlB* coding sequence, or mutants in which a insertion mutation causes a frame shift in the internalin B coding sequence.

One of ordinary skill in the art would also recognize that confirmation of the desired effect of a mutation could be confirmed using one the *in vitro* and *in vivo* assays taught in the specification and/or otherwise known to those of ordinary skill in the art. The ability to infect hepatocytes can be measured directly *in vitro*. Teachings and examples regarding such *in vitro* assays can be found, e.g., at paragraphs [0089] and [0092] and in Examples 9 and 10 of Applicants' specification. *In vivo* methods of assaying for a decreased ability to enter non-phagocytic cells are provided in paragraphs [0093] to [0094], as well as in Example 8.

Similarly, one of ordinary skill in the art would readily recognize that the inventors were in possession of a representative number of mutations that would attenuate *Listeria* for cell-to-cell spread. Applicants teach a number of genes which may be targeted to produce *Listeria* attenuated for cell-to-cell spread (relative to wild type). See, e.g., paragraphs [0133] to [0140]. Guidance regarding methods of producing the mutants are also provided e.g., in paragraphs [0141] to [0142] at pages 38-39 of the specification. As discussed above with respect to *inlB* mutations, mutation of a target gene so as to disrupt the functionality of its gene product and/or expression of that gene product would be routine. For instance, it would be readily apparent to one of ordinary skill in the art that deleting most or all of the *actA* gene, causing a frame shift mutation in the *actA* gene, or generating a termination mutation in the *actA* gene would all be likely to produce the desired effect. In addition, Skoble et al, J. Cell Biology, 150:527-537 (2000) describes a variety of ActA mutants and the effects of such mutations on actin polymerization (a function important for cell-to-cell spread).

Assays for confirming that a given mutant is, in fact, attenuated for cell-to-cell spread are also provided in Applicants' specification and are known to those of ordinary skill in the art. For instance, Applicants' specification provides non-limiting, exemplary *in vitro* assays for assessing whether *Listeria* are attenuated for cell-to-cell spread such as measuring plaque formation on cell monolayers (see, e.g., paragraph [0129] at pages 32 and 33). Assays for assessing the effect of mutations in ActA on actin polymerization *in vitro* and *in vivo* were known in the art as demonstrated in Skoble et al, J. Cell Biology, 150:527-537 (2000).

As indicated in MPEP 2163(II)(A)(2), generally "there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. Information which is well known in the art need not be described in detail in the specification. See, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.* 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986)." Applicants respectfully contend one of ordinary skill in the art would have been readily able to identify a wide variety of mutations for disrupting either expression of any target genes such as *actA* or *inlB* and/or the functionality of the products of such genes and would have recognized Applicants as being in possession of such mutations. Since alternative methods for disrupting the expression or function of such genes would have been so obvious to one of ordinary skill in the art, it is not necessary to recite all such possible mutations or even any particular in order to meet the written description requirement. Applicants contend that, especially in light of the ease with which one of ordinary skill in the art could disrupt given target gene sequences in *Listeria*, the disclosures in the application are more than adequate to provide a representative number of species and meet the written description requirement for the full scope of the pending claims.

Lastly, Applicants wish to note that the Examiner has made a couple of remarks in the Office Action that appear to have been made in error since they do not accurately reflect the prosecution history of the present application. On page 14 of the Office Action, the Examiner has stated, "Applicant asserts 'there is clearly written descriptive support in the specification for gene sequences that possess 70% or greater identity to the claimed sequences' and cites the specification at page 20, lines 28 through page 21, line 27." Applicants have made no such statement during

prosecution of the present application. Similarly, on page 14 of the Office Action, the Examiner states, "The applicant is therefore claiming, as indicated in the prior action, a genus of mutated bacteria solely by their intended effects, without providing any structural or other information by which one skilled in the art could identify the claimed inventions." Applicants note that there has been no prior action on the merits in the present application. Clarification or correction on the record regarding these matters is respectfully requested.

In light of above remarks, Applicants respectfully request that the rejection of claims 22-41 under 35 U.S.C. § 112, first paragraph as failing to comply with the written description requirement be withdrawn.

Claim Rejections under 35 U.S.C. § 102

1. Claims 22-24, 27-30, 32, 37, 38 and 39 are rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Appelberg et al. (Infect. Immun. Feb. 2000 68(2): 912-914).

Applicants respectfully traverse this rejection.

Claim 22, as amended, is directed to an isolated *Listeria* bacterium which is attenuated both for entry into non-phagocytic cells and for cell-to-cell spread relative to wild type, wherein the non-phagocytic cells are hepatocytes. Claim 23 is directed to the bacterium (of claim 22) comprising at least one mutation in one or more genes selected from the group consisting of *actA*, *lplA*, *plcA*, *plcB*, *mpl* and *hly*, wherein the mutation attenuates the bacterium for cell-to-cell spread relative to wild type. In claim 24, the mutation is in *actA*. Claim 27 is directed to the bacterium which is defective with respect to one or more internalins relative to wild type, such that the bacterium is attenuated for entry into the non-phagocytic cells. Claim 28 is directed to the bacterium wherein the one or more internalins comprise internalin B. Claim 29 is directed to the bacterium which comprises at least one mutation in the *inlB* gene that attenuates the bacterium for entry into the non-phagocytic cells. Claim 30 is directed to the bacterium which is defective with respect to ActA relative to wild type, such that the bacterium is attenuated for cell-to-cell spread. In claim 32, the bacterium belongs to the species *Listeria monocytogenes*. Claim 37 is directed to an immunogenic composition

comprising the attenuated *Listeria* bacterium of claim 22. Claim 38 is directed to a composition comprising the bacterium of claim 22 and an adjuvant. Claim 39 is directed to a method of inducing an immune response in a host to an antigen comprising administering to the host an effective amount of a composition comprising the attenuated *Listeria* bacterium of claim 22, wherein the attenuated *Listeria* bacterium comprises a nucleic acid encoding the antigen.

To anticipate a claim, a prior art reference must teach or suggest each and every limitation of the claim. The Appelberg et al. reference does not anticipate the claims (either in their original forms or as amended), because the reference does not teach or suggest each and every element of the claims. Even if the Appelberg et al. reference does report on a *Listeria* strain defective in *actA*, as well as on a separate *Listeria* strain defective in *inlAB*, the Appelberg et al. reference nevertheless does not report a single existing bacterium that it states is attenuated both for entry into non-phagocytic cells *and* for cell-to-cell spread.

As indicated at lines 6-8 of paragraph [0085] of Applicants' specification, "A *Listeria* bacterium that is attenuated for entry into non-phagocytic cells is less able to infect at least one type of non-phagocytic cell from the extracellular environment of the non-phagocytic cell than wild type *Listeria* of the same species." Thus, the attenuation or decreased ability of the claimed *Listeria* to enter non-phagocytic cells is with respect to a particular characteristic of *Listeria*, the ability to enter non-phagocytic cells from the *extracellular* environment, not necessarily with respect to general virulence.

As indicated at lines 3-5 of paragraph [0125] of Applicants' specification, "A *Listeria* bacterium is attenuated for cell-to-cell spread if the *Listeria* bacterium is less able to spread *intercellularly* from one infected cell (a cell comprising the *Listeria* within its cytoplasm) to a neighboring cell." Thus, the attenuation or decreased ability of the claimed *Listeria* to spread cell-to-cell is with respect to a particular characteristic of *Listeria*, the ability to spread intercellularly from one infected cell to another.

The Examiner states at page 15 of the Office Action that the mutant strains taught by the Appelberg et al. reference “were shown to be less virulent than wild-type strains.” Even if this were true, the fact that a strain of *Listeria* is less virulent than wild type or is just said to be “attenuated,” does not, in and of itself, necessarily indicate that the *Listeria* are specifically attenuated for entry into non-phagocytic cells (i.e., less able to infect non-phagocytic cells from the extracellular environment) than wild type or that the *Listeria* are specifically attenuated for entry into hepatocytes (i.e., less able to infect hepatocytes from the extracellular environment) than wild type. Accordingly, it cannot be assumed that *Listeria* that are less virulent than wild type are necessarily attenuated specifically for entry into nonphagocytic cells and/or hepatocytes.

In addition, the Examiner has cited a portion of the Appelberg et al. reference which speculates that “it will be interesting to analyze the characteristics of double mutants defective in both the ActA and the internalin pathways.” This statement clearly confirms that no such mutant had yet been made. Further, it indicates that there was no certainty at the time as to what the characteristics of such a double mutant would be. Importantly, the cited speculative statement in the Appelberg et al. reference does not teach with any certainty that the hypothetical double mutants “defective in both the ActA and the internalin pathways” would necessarily be attenuated for entry into nonphagocytic cells that are hepatocytes. A number of internalin proteins in *Listeria* have been identified such as InlA, InlB, InlC, InlC2, InlD, InlE, InlF, InlG, and InlH (see, e.g., paragraph [0102] at page 25 and paragraph [0106] at page 26), but the internalin proteins do not all, for instance, direct uptake into the same types of cells. Internalin B directs the uptake of *Listeria* by hepatocytes (see, e.g., paragraph [0109] at page 27 of the specification and Lecuit et al., *Infection and Immunity*, 65:5309-5319 (1997)). Internalin A, on the other hand, is known to primarily direct the uptake of *Listeria* into epithelial cells such as those of the intestines (see, e.g., [0108] at pages 26-27 of the specification, Mengaud et al., *Cell*, 84:923-932 (1996), and Lecuit et al., *Infection and Immunity*, 65:5309-5319 (1997)). Thus, *Listeria* defective in an internalin other than internalin B, such as internalin A, are not necessarily attenuated for entry into hepatocytes. Since the speculative “double mutants defective in both the ActA and the internalin pathways” referenced in the

Appelberg et al. reference would not necessarily be attenuated for entry into nonphagocytic cells that are hepatocytes, claim 1 is not anticipated by the Appelberg et al. reference.

Furthermore, the Appelberg et al. reference itself would not necessarily lead one of ordinary skill in the art to have any reasonable expectation that even an *actA* and *inlB* double mutant would be attenuated both for entry into hepatocytes and for cell-to-cell spread. As shown in Figure 1 on page 913 of the Appelberg et al. reference, the reference teaches that the growth of the InlAB deficient strain (“949”) in the livers of the neutrophil-depleted mice was generally unaffected relative to a wild type strain (“10403S”). Furthermore, one of ordinary skill in the art would be skeptical as to the relevance of the data in the Appelberg et al. reference to the *in vivo* situation in normal animals, since the key data in the Appelberg et al. reference is from neutrophil-depleted mice. The Appelberg et al. reference does not provide data that would necessarily be convincing to one of ordinary skill in the art that an *actAinlB* double mutant would actually be attenuated for entry into hepatocytes *in vivo* in a normal animal.

As indicated above, Applicants contend that the Appelberg et al. reference does not teach *Listeria* attenuated both for entry into non-phagocytic cells (or hepatocytes) and for cell-to-cell spread relative to wild type. However, even if the Appelberg et al. reference did teach such *Listeria*, the Appelberg et al. reference would not, *by itself*, anticipate claim 22 since the Appelberg et al. reference does not, by itself, teach how to make the hypothetical “double mutants defective in both the ActA and the internalin pathways.” Rather, the authors of the Appelberg et al. reference indicate that the strain deleted in the *actA* gene and the strain with the defective *inlAB* operon were supplied to the authors by others (see, e.g., the third paragraph of page 912 of Appelberg et al.). The Appelberg et al. reference itself provides absolutely no guidance as to how to make the double mutants, and, in fact, the reference indicates none was actually made or tested by the authors. Thus, the Appelberg et al. reference is not, by itself, enabling for “double mutants defective in both the ActA and the internalin pathways.”

In light of the foregoing remarks, Applicants respectfully request that the rejection of claims 22-24, 27-30, 32, 37, 38 and 39 under 35 USC § 102 be withdrawn.

2. Claims 22-24, 27-30, 32, 37, 38 and 39 are rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Drevets (Infect. Immun. Jan. 1998. 66(1): 232-238).

Applicants respectfully traverse this rejection.

To anticipate a claim, a prior art reference must teach or suggest each and every limitation of the claim. The Drevets reference does not anticipate claims 22-24, 27-30, 32, 37, 38 and 39 (either in their original forms or as amended), because the reference does not teach or suggest each and every element of the claims. Even if the Drevets reference does report on a *Listeria* strain defective in the *actA* gene, as well as on a separate *Listeria* strain defective in the *inlB* gene, the reference nevertheless does not report even a single bacterium that it states is attenuated for *both* entry into non-phagocytic cells *and* cell-to-cell spread.

As indicated at lines 6-8 of paragraph [0085] of Applicants' specification, "A *Listeria* bacterium that is attenuated for entry into non-phagocytic cells is less able to infect at least one type of non-phagocytic cell from the extracellular environment of the non-phagocytic cell than wild type *Listeria* of the same species." Thus, the attenuation or decreased ability of the claimed *Listeria* to enter non-phagocytic cells is with respect to a particular characteristic of *Listeria*, the ability to enter non-phagocytic cells from the *extracellular* environment, not necessarily with respect to general virulence.

As indicated at lines 3-5 of paragraph [0125] of Applicants' specification, "A *Listeria* bacterium is attenuated for cell-to-cell spread if the *Listeria* bacterium is less able to spread *intercellularly* from one infected cell (a cell comprising the *Listeria* within its cytoplasm) to a neighboring cell." Thus, the attenuation or decreased ability of the claimed *Listeria* to spread cell-to-cell is with respect to a particular characteristic of *Listeria*, the ability to spread intercellularly from one infected cell to another.

The Examiner indicates at page 16 of the Office Action that the mutant strains taught by the Drevets reference "were shown to be less virulent than wild-type strains." Even if this were true, the fact that a strain of *Listeria* is less virulent than wild type or is just said to be "attenuated," does

not, in and of itself, necessarily indicate that the *Listeria* are specifically attenuated for entry into non-phagocytic cells (i.e., less able to infect non-phagocytic cells from the extracellular environment) than wild type or that the *Listeria* are specifically attenuated for entry into hepatocytes (i.e., less able to infect hepatocytes from the extracellular environment) than wild type. Accordingly, it cannot be assumed that *Listeria* that are less virulent than wild type are necessarily attenuated specifically for entry into nonphagocytic cells and/or hepatocytes.

In light of the foregoing remarks, Applicants respectfully request that the rejection of claims 22-24, 27-30, 32, 37, 38 and 39 under 35 USC § 102 be withdrawn.

Claim Rejection under 35 U.S.C. § 103

Claim 31 stands rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Appelberg et al. (Infect. Immun. Feb. 2000, 68(2): 912-914).

Applicants respectfully traverse this rejection.

Claim 31, as amended, is directed to a *Listeria* bacterium which comprises at least one mutation in both *actA* and *inlB*, wherein the mutation in *actA* attenuates the bacterium for cell-to-cell spread relative to wild type and the mutation in *inlB* attenuates the bacterium for entry into hepatocytes relative to wild type.

To establish a prima facie case of obviousness, the prior art reference must teach or suggest each and every claim limitation. Applicants respectfully submit that claim 31 (both in the original form and as amended) is not obvious over the Appelberg et al. reference, because the Appelberg et al. reference does not teach or suggest each and every element of claim 31.

The Examiner has cited a portion of the Appelberg et al. reference which speculates that “it will be interesting to analyze the characteristics of double mutants defective in both the ActA and the internalin pathways.” This statement not only confirms no such mutant had yet been made, but, importantly, also fails to indicate with any specificity and certainty exactly which internalin

pathway(s) the speculated mutants would be defective in and in exactly what manner the mutants would be made defective. The speculative statements in the Appelberg et al. reference do not teach with any specificity or certainty that the double mutants “defective in both the ActA and the internalin pathways” would necessarily comprise at least one mutation in both *actA* and *inlB*, where the mutation in *actA* attenuates the bacterium for cell-to-cell spread and the mutation in *inlB* attenuates the bacterium for entry into non-phagocytic cells. The speculative statements in the Appelberg et al. reference also do not teach with any specificity or certainty that the double mutants would necessarily comprise at least one mutation in both *actA* and *inlB*, where the mutation in *actA* attenuates the bacterium for cell-to-cell spread and the mutation in *inlB* attenuates the bacterium for entry into non-phagocytic cells, wherein the non-phagocytic cells are hepatocytes.

Even if the *Listeria* were to be “defective in...the internalin pathways,” this would not necessarily indicate to one of ordinary skill in the art that the bacteria were defective in the internalin B pathway or that the bacteria would comprise a mutation specifically in the *inlB* gene. A number of internalin proteins in *Listeria* had been identified at the time of Applicants filing such as InlA, InlB, InlC, InlC2, InlD, InlE, InlF, InlG, and InlH (see, e.g., paragraph [0102] at page 25 and paragraph [0106] at page 26 of Applicants’ specification). The Appelberg et al. reference itself refers to both internalin A and internalin B, not just internalin B (see, e.g., the abstract and first paragraph of the Appelberg et al. reference). In addition, The Appelberg et al. reference itself indicates uncertainty as to which products are actually involved in the internalization by hepatocytes when it states in the same paragraph bridging pages 913-914 that contained the statement cited by the Examiner, “In addition, there may be other gene products involved in the internalization by non-phagocytic cells such as the hepatocyte.”

Furthermore, just as the Appelberg et al. reference does not teach or suggest with any certainty that the speculated double mutants would comprise at least one mutation in *inlB*, the reference does not teach or suggest with any certainty that the speculated double mutants would necessarily comprise a mutation in *inlB* that attenuates the bacterium for entry into hepatocytes. The statement in the Appelberg et al. reference cited by the Examiner which speculates that “it will be interesting to analyze the characteristics of double mutants defective in both the ActA and the

internalin pathways,” indicates that there was no certainty at the time as to what the characteristics of such a double mutant would be. The speculation in the paragraph bridging pages 913-914 to which the Examiner points is based primarily on the data from neutrophil-depleted mice, an artificial system which would allow for unnatural levels of infection to occur. Furthermore, as shown in Figure 1 on page 913 of the Appelberg et al. reference, the reference teaches that the growth of the InlAB deficient strain (“949”) in the livers of the neutrophil-depleted mice was generally unaffected relative to a wild type strain (“10403S”). Thus, the Appelberg et al. reference does not actually provide any data that shows that the InlAB deficient strain was attenuated for entry into hepatocytes. Rather, the “hypothesis that such an invasion of hepatocytes by the ActA mutant is mediated by the InlAB-induced internalization of the bacterial directly by the hepatocytes” on page 913 is just that – a hypothesis. If it was obvious to one of ordinary skill in the art from the data in the Appelberg reference that the speculated double mutants would be attenuated for both cell-to-cell spread and entry into hepatocytes, then the reference would not have indicated that the characteristics of the double mutants would be “interesting to analyze.” That which is obvious is not “interesting.”

In addition, the claimed bacteria are not obvious over the Appelberg et al. reference, because Applicants have shown that the claimed bacteria have unexpected properties which one of ordinary skill in the art would not have predicted, even in view of the Appelberg et al. reference. As noted in the first column of page 13832 of Brockstedt et al., “*Listeria*-based cancer vaccines that segregate immunogenicity from toxicity,” *PNAS*, 101:13832-13837 (2004), “the practical utility of live attenuated vaccines relies on achieving a proper balance between the virulence/toxicity and immunogenicity of the vaccine.” Even *if* it were obvious that certain mutations would result in decreased ability to enter hepatocytes and decreased ability for cell-to-cell spread, it would not have been obvious prior to Applicants invention that *Listeria* comprising those mutations would still be highly immunogenic despite their decreased toxicity. Applicants have demonstrated in experiments disclosed in the specification, as well as elsewhere, that although *Listeria* that are attenuated for entry into hepatocytes (e.g., through a mutation in *inlB*) and for cell-to-cell spread (e.g., through a mutation in *actA*) relative to wild type have diminished toxicity, the *Listeria* nonetheless maintain

sufficient immunopotency to be useful as immunotherapeutics. See, e.g., Examples 5 and 6 and Figures 3-5 of the specification and Brockstedt et al., *PNAS*, 101:13832-13837 (2004), including Figure 2, as well as Figure 5A of the “Supporting Information” available for the Brockstedt et al. (2004) online at www.pnas.org/cgi/content/full/0406035101/DC1. In addition, data in Table 1 of the “Supporting Information” for Brockstedt et al. (2004) indicate that immunization with 0.1 LD₅₀ of the *actA**inlB* *L. monocytogenes* mutant “CS-L0001,” elicits approximately the same level of protective immunity (see “Log protection” of Table 1) as 0.1 LD₅₀ wild type *L. monocytogenes* does against subsequent challenge with 2 x LD₅₀ wild type *L. monocytogenes* 28 days post the primary vaccination, despite the decreased toxicity of the mutant. Nothing in the Appelberg et al. reference teaches or suggests that “double mutants defective in both the ActA and the internalin pathways” would be immunopotent enough to be suitable as immunotherapeutics, including as cancer immunotherapeutics. Nothing in the reference teaches or suggests that an *actA* and *inlB* double mutant strain of *Listeria* would maintain immunogenicity, despite the decrease in toxicity.

The Examiner has stated that “one of ordinary skill in the art would have been motivated to produce a mutant deficient in both ActA and inlB in order to find a way of treating such a virulent infection.” Applicants respectfully disagree. The motivation suggested by the Examiner presupposes that it would have been obvious to one of ordinary skill in the art that an *actA* and *inlB* double mutant would be both attenuated for cell-to-cell spread and entry into hepatocytes and that such a mutant with decreased infectivity would nonetheless maintain its immunogenicity and be suitable as an immunotherapeutic. As described above, these presuppositions are not supported by the reference cited by the Examiner, nor common sense, and thus the Examiner’s *prima facie* argument for obviousness is not proper. Applicants note that nothing in the Appelberg et al. reference suggests the use of *Listeria* in vaccines or immunotherapeutics and one of ordinary skill in the art looking to make an immunotherapeutic or vaccine, e.g., for listeriosis, would have a variety of potential options for producing such an immunotherapeutic or vaccine. A vaccine for listeriosis, for instance, could in theory, at least, be a peptide vaccine, a DNA vaccine, inactivated *Listeria*, or attenuated *Listeria*. Even if one of ordinary skill in the art would have been motivated to make an attenuated *Listeria*, a variety of virulence genes in *Listeria* had been identified, including, but not

limited to those referred to in the Appelberg et al. reference. The Examiner has not provided any rationale as to why one of ordinary skill in the art would be motivated to select a form of the vaccine that was an *actAinlB* double mutant of *Listeria* over another known, attenuated form of *Listeria*. The Examiner has not provided any reasoning as to why one of ordinary skill in the art would have been motivated to pursue the specific claimed form of *Listeria* as a vaccine or immunotherapeutic. Applicants respectfully contend that the asserted motivation for making claimed *actA/inlB* double mutant for use as a vaccine or immunotherapeutic comes only from Applicants' own disclosure, and as such, represents impermissible hindsight.

For the foregoing reasons, Applicants respectfully request that the rejection of claim 31 over Appelberg et al. under 35 USC § 103(a) be withdrawn.

Supplemental Information Disclosure Statement filed November 2, 2006

Applicants would like to thank the Examiner for returning the Form PTO/SB/08a/b for the Supplemental Information Disclosure Statement filed November 2, 2006, with the Non-Final Office Action mailed January 31, 2007. However, the Form PTO/SB/08a/b was not initialed by the Examiner. Applicants kindly request that the Examiner provide a copy of the initialed Form PTO/SB/08a/b at the Examiner's earliest convenience.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, Applicants petition for any required relief including extensions of time and authorize the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. **282172002900**. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: July 31, 2007

Respectfully submitted,

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